

8EHQ-0504-15609 S

# CYTEC

Cytec Industries Inc.  
5 Garret Mountain Plaza  
West Paterson, NJ 07424

May 17, 2004

Document Processing Center (7407M)  
EPA East - Room 6428  
ATTN: SECTION 8(E) COORDINATOR  
U.S. Environmental Protection Agency  
Office of Pollution Prevention and Toxics (OPPT)  
1201 Constitution Avenue, NW  
Washington, DC 20460-0001

PHONE# (202) 564-8930

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MR 275447



Dear Sir/Madam:

The purpose of this letter is to inform you under Section 8(e) of TSCA of the results obtained from a study entitled "CT-756-02: Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions" conducted with a commercial product with the following variable composition:

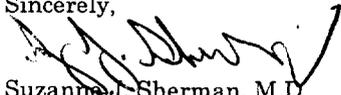
CAS# 68608-26-4	Sulfonic acids, petroleum, sodium salts	50-80%
CAS# 71-36-3	Butanol	6-16%
CAS# Unassigned	Hydrocarbon Mixture	6-8%
CAS# Unassigned	Alcohol	3-7%

The results indicate that this product has a 96-hr LC50 to Rainbow Trout of 0.89 mg/L, which renders the product as highly toxic. It is our opinion, that active ingredient (CAS# 68608-26-4), is the likely component causing this adverse effect.

The final report, which does NOT contain any CBI information, is enclosed for your files.

Please direct all communications on this subject to Patricia Ann Vernon, Manager, Regulatory Toxicology at the address above or call her at (973) 357-3375.

Sincerely,

  
Suzanne J. Sherman, M.D.  
Corporate Medical Director

Cc: R. Deskin  
S. Fleming  
T. Mesevage

**COMPANY SANITIZED**

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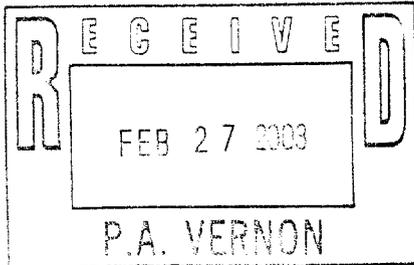
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**STUDY TITLE**

CT-756-02: Acute Toxicity To Rainbow Trout,  
*Oncorhynchus mykiss*,  
Under Static Test Conditions



**DATA REQUIREMENT**

OECD Guideline 203

**AUTHORS**

Kerri Larsen  
Scott Glover

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**STUDY INITIATION DATE**

December 4, 2002

**STUDY COMPLETION DATE**

February 24, 2003

**SPONSOR**

Cytec Industries  
Five Garret Mountain Plaza  
West Paterson, NJ 07424

**PERFORMING LABORATORY**

Toxikon Corporation  
106 Coastal Way  
Jupiter, Florida 33477

**LABORATORY PROJECT ID**

02J0053b

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**STATEMENT OF GOOD LABORATORY PRACTICES COMPLIANCE**

Test Substance: CT-756-02

Title: CT-756-02: Acute Toxicity to Rainbow Trout,  
*Oncorhynchus mykiss*, Under Static Test Conditions

This study was conducted in accordance with and fully complies with published Good Laboratory Practices (GLP) regulations for tests of substances as promulgated by the OECD Guidelines for Testing of Chemicals (OECD, 1998). The sections of the regulations not performed by or under the direction of Toxikon Corporation, exempt from this Good Laboratory Practice Statement, included characterization of the test article and its mixture with carriers, Section 6.2.

Scott Glover  
Scott Glover  
Study Director  
Toxikon Corporation

02/24/03  
Date

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**STATEMENT OF QUALITY ASSURANCE**

Test Substance: CT-756-02

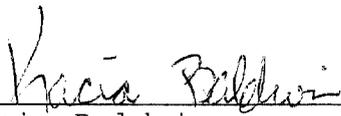
Title: CT-756-02: Acute Toxicity to Rainbow Trout,  
*Oncorhynchus mykiss*, Under Static Test Conditions

This study was examined for conformance with Good Laboratory Practices as published by the Organization For Economic Co-Operation And Development (OECD). The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

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<u>TYPE OF AUDIT</u>	<u>DATE OF AUDIT</u>	<u>DATE FINDINGS REPORTED TO THE STUDY DIRECTOR AND TO MANAGEMENT</u>
In-Life Audit:	02/10/03	02/12/03
Study Data Review:	02/21/03	02/21/03
Draft Report Review:	02/21/03	02/21/03
Final Report Review:	02/24/03	02/24/03

  
\_\_\_\_\_  
Kacia Baldwin  
Quality Assurance Officer  
Toxikon Corporation

02/24/03  
\_\_\_\_\_  
Date

**LIST OF SCIENTIFIC PERSONNEL**

Test Substance: CT-756-02

Title: CT-756-02: Acute Toxicity to Rainbow Trout,  
*Oncorhynchus mykiss*, Under Static Test Conditions

Study Director: Scott Glover

Biologists: Shivanna Mahabir  
Kerri Larson

Aquaculturist: Matthew W. Bolt

Manager of Ecotoxicology: Scott Glover

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**SUMMARY**

Sponsor: Cytec Industries  
Five Garret Mountain Plaza  
West Paterson, NJ 07424

Study Director: Scott Glover

Location of Study: Toxikon Corporation  
106 Coastal Way  
Jupiter, Florida 33477

Location of Raw Data and Final Report: Cytec Industries  
West Paterson, New Jersey

Test Substance: CT-756-02; Lot #04302002

Test Species: Juvenile rainbow trout, *Oncorhynchus mykiss*; 4.3 ± 0.20 cm average total length and 0.5597 ± 0.08233 g average wet weight.

Source of Organisms: Thomas Fish Company,  
Anderson, CA

Condition at Study Initiation: Rainbow trout appeared to be in good physical condition at study initiation; mortality was 0% during the 7-day period prior to test initiation.

Dilution Water: Filtered freshwater with an initial conductivity, hardness and alkalinity of 343 µS, 54 mg/L as CaCO<sub>3</sub>, and 16 mg/L as CaCO<sub>3</sub>, respectively; initial test temperature range of 14.1 to 14.3°C.

Nominal Concentrations: Control, Solvent Control, 0.016, 0.080, 0.40, 2.0, and 10 mg wm/L

Test Dates: February 10, 2003 to February 14, 2003

Study Length: 96 hours

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Results:

The 96-hour  $LC_{50}$  was 0.89 mg wm/L with unreliable 95% confidence limits. The no-observable-effect concentration (NOEC) was 0.40 mg wm/L based on the lack of statistically significant mortality at this testing concentration.

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Toxikon Corporation  
Study No. 02J0053b

**1.0 INTRODUCTION**

A static freshwater toxicity test was conducted at Toxikon Corporation, Jupiter, Florida, to determine the acute toxicity of CT-756-02 to rainbow trout, *Oncorhynchus mykiss*. The criterion for effect was death. Results of the test are expressed as a 96-hour median lethal concentration (LC<sub>50</sub>), the concentration of CT-756-02 calculated to result in death to 50 percent of the test population at the specified time.

**2.0 MATERIALS AND METHODS**

**2.1 TEST SUBSTANCE**

The test substance, CT-756-02 (Lot No.04302002) was received at Toxikon Corporation on November 27, 2002 from Cyttec Industries in a 300 mL clear glass bottle labeled "CT #756-02; Quantity Shipped 250 mL; Lot # 04302002; Expiration Date: 11/2004." The test substance was a dark brown liquid stored at room temperature. The MSDS reported the compound to be dispersible in water.

Test concentrations are reported as milligrams (mg) of CT-756-02 as whole material (wm) per liter (L) of freshwater.

**2.2 TEST SPECIES**

Juvenile rainbow trout, *Oncorhynchus mykiss*, for the definitive test, were received on January 28, 2003 at Toxikon Corporation from Thomas Fish Company, Anderson, California. *O. mykiss* were maintained in filtered laboratory freshwater at a temperature of 11.1 to 15.4°C during the 12-day period prior to test initiation. The test organisms used were from lot number OM 03-1-28. *O. mykiss* were maintained on a diet of salmon starter (Ziegler

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Brothers, Inc., Gardners, PA), but were not fed for at least 24 hours prior to test initiation or during the test. The test organisms appeared to be in good physical condition at test initiation. Mortality during the 7-day period prior to test initiation was zero percent.

*O. mykiss* used for the definitive test ranged from 3.9 to 4.5 centimeters (cm) total length (mean and standard deviation =  $4.3 \pm 0.20$  cm) and from 0.3840 to 0.6641 grams (g) wet weight (mean and standard deviation =  $0.5597 \pm 0.08233$  g) as measured from the control fish at test termination. Loading was calculated to be 0.62 g of fish tissue per liter of test solution.

### **2.3 TEST WATER**

The dilution water was a moderately hard freshwater. The water originated from the Town of Jupiter and was treated by vigorous aeration, filtered to 20 micrometers, passed through activated carbon, and re-aerated prior to use. The dilution water, at test initiation, possessed a hardness and alkalinity of 54 and 16 mg/L as calcium carbonate (CaCO<sub>3</sub>) respectively, and specific conductivity of 343 microSiemens (μS).

Chemical characterization of a recent representative batch of freshwater is presented in Appendix A.

### **2.4 TEST METHODS**

Methods used for the 96-hour static test were those described in Toxikon Corporation's test protocol entitled: "CT-756-02: Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions".

An initial solubility test of CT-756-02 was conducted in Toxikon's in-house dilution water that proved the compound to be dispersible at a concentration of approximately 1,900 mg wm/L. Additionally, the compound was soluble in dimethylformamide (DMF) at a concentration of approximately 100,000 mg wm/L. Upon spiking this stock into laboratory freshwater at a concentration of 100 mg wm/L, a slight film on the surface was observed. After discussions with the Sponsor, it was decided to use dimethylformamide as a carrier in order to dose the dilution water and achieve usable range-finding and definitive test concentrations.

A 96-hour static range-finding test was conducted at nominal concentrations of 0.010, 0.10, 1.0, 10, and 100 mg wm/L prior to performing the definitive test. Two fish were tested at each concentration, control, and solvent control. After 96 hours of exposure, there was zero percent mortality in the control, solvent control, and 0.010 mg wm/L testing concentration. Testing concentrations 0.10 and 1.0 mg wm/L both yielded 50% mortality. Testing concentrations 10 and 100 mg wm/L both yielded 100 percent mortality. Fish utilized in the range-finding test averaged 5.1 cm in total length and 1.0191 g in wet weight as measured from the control fish at test termination. The range-finding results yielded a calculated 96-hour LC<sub>50</sub> value of 0.32 mg wm/L. Based upon these preliminary results, nominal test concentrations of 0.016, 0.080, 0.40, 2.0 and 10 mg wm/L were selected for the definitive test.

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Study No. 02J0053b

Testing stocks for the definitive exposure consisted of a primary (1°) and secondary (2°) stock. The primary stock (100,000 mg wm/L in DMF) was prepared by adding 1.0007 grams (g) of CT-756-02 into a 10.0 mL volumetric flask and diluting to volume with DMF. The secondary stock (1,000 mg wm/L in DMF) was prepared by adding 100 µL of primary stock into a 10.0 mL volumetric flask and diluting to volume with DMF. Test solutions used in the definitive test were prepared by spiking appropriate volumes of dilution water to yield nominal definitive testing concentrations of 10, 2.0, 0.40, 0.080, and 0.016 mg wm/L. A dilution water control and solvent control were also maintained concurrently with the test solutions.

The 96-hour definitive toxicity test was initiated on February 10, 2003 with the impartial addition of rainbow trout, by ones and twos, to all test chambers until 10 rainbow trout were distributed to each replicate test chamber after initial water quality parameters were measured. Each test treatment and control was initiated with two replicates resulting in a total of 20 fish per treatment and control. The test chambers were rectangular glass tanks (43-cm length x 23-cm width x 14-cm height) containing 9.0 L of dilution water or test solution and providing a final water depth of approximately 10 centimeters. All test chambers were covered throughout the exposure period to reduce evaporation. The test chambers were positioned in a water bath under fluorescent lighting regulated to a photoperiod of 16 hours light and 8 hours darkness. The light intensity ranged between 3.7 and 4.7 micromols per square meter per second as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a  $2\pi$  quantum sensor at the surface of the test solutions.

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Survival of rainbow trout was monitored daily. The fish were also monitored for any abnormalities in their behavior or appearance.

Water quality (i.e., temperature, pH and dissolved oxygen concentration) was measured daily in each replicate. The diurnal range of the water bath temperature was continuously monitored using a minimum/maximum thermometer and recorded daily. Specific conductivity, total alkalinity, and total hardness of the dilution water were measured at test initiation. Specific conductivity was measured using a Corning Check-mite digital conductivity meter model CD-30. Water hardness and alkalinity were determined by EDTA and potentiometric titration, respectively (APHA et al., 1992). Dissolved oxygen concentrations and test chamber temperatures were determined using a YSI Model 58 oxygen meter utilizing a membrane electrode. The pH was measured with an Oakton pH Testr2 Waterproof meter.

## **2.5 STATISTICAL ANALYSES**

The 24, 48, 72, and 96-hour LC<sub>50</sub> values were calculated based on the number of dead fish observed at the specified time. The LC<sub>50</sub> values and 95 percent confidence limits were estimated by an EPA computer program (Stephan, 1977). In addition to the LC<sub>50</sub> values, a no-observable-effect-concentration (NOEC) was calculated using the statistical program ToxCalc (ToxCalc, Version 5.0). Statistical differences were determined at a probability level of 0.05.

## 2.6 ARCHIVES

The final report and all data related to this study will be archived at Cytec Industries, West Paterson, New Jersey.

## 3.0 RESULTS AND DISCUSSION

Mortality of rainbow trout exposed for 96 hours to CT-756-02 was 0% in the solvent control, 0.016, 0.080, and 0.40 mg wm/L test concentrations. There was 5% mortality in the control and 100% mortality in the 2.0, and 10 mg wm/L test concentrations (Table 1). The 24-hour LC<sub>50</sub> was calculated to be 1.1 mg wm/L with 95% confidence limits of 0.85 and 1.3 mg wm/L. The 48, 72 and 96-hour LC<sub>50</sub> values were calculated to be 0.89 mg wm/L (95% confidence limits are not reliable) (Stephan 1977) (Table 5). The NOEC was 0.40 mg wm/L (nominal concentration) based on a lack of significant mortality at this test concentration.

The test temperature during the 96-hour exposure ranged from 13.9 to 14.7°C (Table 2). The dissolved oxygen concentration in the controls and all test solutions at test initiation ranged from 10.0 to 10.3 mg/L (97 to 100 percent of saturation). The dissolved oxygen concentrations ranged from 7.0 to 8.7 mg/L (68 to 85 percent of saturation) in all test chambers for the remainder of the test (Table 3). Initial pH values of the control and the test solutions ranged from 7.4 to 7.5; the pH values ranged from 6.9 to 7.1 for the remainder of the test (Table 4).

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#### 4.0 PROTOCOL DEVIATIONS

There were two deviations from the test protocol during the conduct of this test.

1) As stated in Section 11.3 of the test protocol, the fish used in the test should range between 4 and 6 centimeters in total length. However, one fish from the control was 3.9 cm in total length.

2) As stated in Section 12.2 of the test protocol, the temperature will be regulated to maintain  $15 \pm 1^{\circ}\text{C}$ . However, the temperature was  $13.9^{\circ}\text{C}$  in replicate B of 0.016 mg wm/L and both replicates of 0.080 mg wm/L on Day 1 of the test.

These deviations were minor and, in the scientific opinion of the Study Director, did not affect the validity of the test results.

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### REFERENCES

- American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environmental Federation (WEF). 1992. Standard Methods for the Examination of Water and Wastewater, 18th Edition.
- OECD Guideline for Testing of Chemicals, Method 203 "Fish Acute Toxicity Test", 1992.
- Stephan, C.E., 1977. Methods for Calculating an LC50: F.L. Mayer and J.L. Hamelink, eds., Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, American Society for Testing and Materials, Philadelphia, Pennsylvania. pp. 65-84.
- Toxcalc Comprehensive Toxicity Data Analysis and Database Software Version 5.0, 1996.

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Table 1. Mortality of Rainbow Trout, *Oncorhynchus mykiss*,  
 During a 96-Hour Exposure to CT-756-02 Under Static  
 Test Conditions

Nominal Concentrations (mg wm/L)	Cumulative Number Dead (Percent Mortality)			
	24 Hr	48 Hr	72 Hr	96 Hr
Control	0 (0)	1 <sup>c</sup> (5)	1 (5)	1 (5)
Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)
0.016	0 (0)	0 (0)	0 (0)	0 (0)
0.080	0 (0)	0 (0)	0 (0)	0 (0)
0.40	0 (0)	0 (0)	0 (0)	0 (0)
2.0	18 <sup>a</sup> (90)*	20 (100)*	20 (100)*	20 (100)*
10	20 <sup>b</sup> (100)*	20 (100)*	20 (100)*	20 (100)*

\* Statistically significant compared to the survival of the control animals

<sup>a</sup> One fish dark and two fish at surface

<sup>b</sup> Slightly cloudy

<sup>c</sup> One fish not found

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Table 2. Daily Temperature Values During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to CT-756-02

Exposure Period (Hrs)	Temperature Range <sup>a</sup> (°C)
0	14.1 - 14.3
24	13.9 - 14.4
48	14.2 - 14.7
72	14.0 - 14.4
96	14.2 - 14.4

<sup>a</sup> Daily temperature values reported are the ranges of temperature taken in all test chambers each day.

Note: The diurnal temperature of the water bath ranged from 14.1 to 15.6°C as continuously measured by a minimum/maximum thermometer during the test.

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Table 3. Dissolved Oxygen Concentrations During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to CT-756-02

Nominal Concentrations (mg wm/L)	R E P	Dissolved Oxygen Concentration (mg/L)				
		0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
Control	A	10.0	7.8	8.1	8.1	8.2
	B	10.0	7.7	7.9	8.0	8.0
Solvent Control	A	10.0	7.7	8.1	7.9	7.9
	B	10.1	7.5	7.9	7.6	7.7
0.016	A	10.1	7.3	7.5	7.5	7.3
	B	10.2	7.5	7.8	7.6	7.6
0.080	A	10.1	7.4	7.6	7.3	7.4
	B	10.2	7.1	7.0	7.2	7.4
0.40	A	10.1	7.5	7.7	7.6	7.6
	B	10.1	7.6	7.5	7.5	7.4
2.0	A	10.3	8.0	8.5	---	---
	B	10.2	8.0	8.4	---	---
10	A	10.2	8.7	---	---	---
	B	10.2	8.6	---	---	---

--- Indicates that DO was not measured due to 100% mortality.

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Table 4. pH Values During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to CT-756-02

Nominal Concentrations (mg wm/L)	R E P	pH				
		0 Hr	24 Hr	48 Hr	72 Hr	96 Hr
Control	A	7.4	7.0	7.1	7.1	7.1
	B	7.4	7.0	7.1	7.1	7.1
Solvent Control	A	7.4	7.0	7.0	7.1	7.1
	B	7.4	7.0	6.9	7.0	7.1
0.016	A	7.4	6.9	6.9	7.0	7.1
	B	7.4	6.9	6.9	7.1	7.1
0.080	A	7.5	6.9	7.0	7.0	7.1
	B	7.5	6.9	6.9	7.0	7.1
0.40	A	7.5	6.9	6.9	7.0	7.1
	B	7.5	7.0	6.9	7.0	7.1
2.0	A	7.5	7.0	7.0	---	---
	B	7.5	7.0	7.1	---	---
10	A	7.5	7.1	---	---	---
	B	7.5	7.1	---	---	---

--- Indicates that pH was not measured due to 100% mortality.

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Table 5. Calculated LC<sub>50</sub> Values for Rainbow Trout, *Oncorhynchus mykiss*, Exposed to CT-756-02 Under Static Conditions

Exposure Period (Hr)	LC <sub>50</sub> (mg wm/L)	95-Percent Confidence Limits (mg wm/L)
24	1.1	0.85 to 1.3
48	0.89	---
72	0.89	---
96	0.89	---

--- 95% Confidence Limits were not reliable

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Toxikon Corporation  
Study No. 02J0053b

APPENDIX A  
DILUTION WATER CHARACTERIZATION

**LABORATORY FRESHWATER CHARACTERIZATION<sup>a</sup>**

<u>Parameter</u>	<u>Concentration<sup>b</sup></u>	<u>Historical Range<sup>c</sup></u>
Aluminum	0.051 mg/L	ND - 1.16 mg/L
Arsenic	<0.010 mg/L	ND
Beryllium	<0.0040mg/L	ND - 0.028 mg/L
Bromide	4.1 mg/L	ND - 30 mg/L
Cadmium	<0.0050mg/L	ND - 0.028 mg/L
Calcium	28 mg/L	8.89 - 28.0 mg/L
Chloride	81 mg/L	60 - 110 mg/L
Chromium (hexavalent)	<0.050 mg/L	ND
Chromium (total)	<0.010 mg/L	ND
Cobalt	<0.010 mg/L	ND - 0.029 mg/L
Copper	<0.010 mg/L	ND - 0.064 mg/L
Fluoride	<0.050 mg/L	ND - 0.826 mg/L
Iodide	<1.0 mg/L	ND
Iron	<0.10 mg/L	ND - 1.74 mg/L
Lead	<0.0050mg/L	ND
Manganese	<0.010 mg/L	ND
Magnesium	3.0 mg/L	0.789 - 9.91 mg/L
Mercury	0.00059mg/L	ND - 0.00059 mg/L
Molybdenum	<0.020 mg/L	ND - 0.054 mg/L
Nickel	<0.020 mg/L	ND
Potassium	1.8 mg/L	1.14 - 5.50 mg/L
Selenium	<0.0050mg/L	ND
Silver	<0.0050mg/L	ND
Sodium	49 mg/L	27.4 - 74.0 mg/L
Tin	<0.30 mg/L	ND
Zinc	0.091 mg/L	ND - 0.091 mg/L
Ammonia (as N)	<0.040 mg/L	ND - 0.676 mg/L
Cyanide (total)	<0.010 mg/L	ND
Nitrates (as N)	0.90 mg/L	ND - 1.38 mg/L
Nitrites (as N)	<0.030 mg/L	ND - 0.062 mg/L
Phosphates (total)	<0.05 mg/L	ND - 0.12 mg/L
Sulfide	<1.0 mg/L	ND
Sulfate	29 mg/L	15 - 52 mg/L
TDS	230 mg/L	150 - 552 mg/L
TOC	<1.0 mg/L	ND - 6.0 mg/L
TSS	<1.0 mg/L	ND - 16 mg/L
COD	5.0 mg/L	ND - 54.6 mg/L
Total organophosphorus pesticides	<1.0 µg/L	ND
Total chlorinated herbicides	<10 µg/L	ND
Total organochlorine pesticides	<0.040 µg/L	ND
PCBs	<0.20 µg/L	ND

<sup>a</sup> The characterized freshwater is carbon-treated Jupiter, Florida, town water which is aerated following carbon treatment.

<sup>b</sup> Sample of freshwater collected August 05, 2002.

<sup>c</sup> Historical range for laboratory freshwater.

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APPENDIX B  
PROTOCOL AND AMENDMENTS

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Test Protocol

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**CT-756-02: Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions**

STUDY NUMBER: 02700536

This protocol complies with  
OECD Guideline Number 203

TOXIKON CORPORATION  
JUPITER, FLORIDA

PROTOCOL NUMBER: E103

### 1.0 TITLE

CT-756-02: Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions

### 2.0 OBJECTIVE

The objective of this test is to determine the 96-hour LC<sub>50</sub> of the test substance to Rainbow Trout under static test conditions. Mortality will be used to evaluate the acute toxicity of the test substance. Any sublethal effects of the test substance on the test species will also be noted. For substances with limited solubility in the test medium, it may not be possible to quantitatively determine the LC<sub>50</sub>.

### 3.0 JUSTIFICATION FOR SELECTION OF METHOD OF EXPOSURE

An acute (96-hour) toxicity test is used to measure the effects of a brief chemical exposure to the species. A static system is used when the test substance is not expected to change or deleteriously affect water quality, both of which could bias test results. For test substances that are expected to be unstable under static test conditions, testing should be conducted under dynamic test conditions.

### 4.0 STUDY SPONSOR

Cytec Industries  
Five Garret Mountain Plaza  
West Paterson, NJ 07424  
TEL: (973) 357-3375 FAX: (973) 357-3057  
Sponsor Representative: Patti Vernon

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#### 5.0 TESTING FACILITY

Toxikon Corporation  
106 Coastal Way  
Jupiter, Florida 33477  
TEL: (561) 575-2477 FAX: (561) 575-2497  
Study Director: Scott Glover

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#### 6.0 PROPOSED SCHEDULE

PROPOSED EXPERIMENTAL START DATE: October 2002  
PROPOSED EXPERIMENTAL COMPLETION DATE: November 2002  
PROPOSED DRAFT REPORT SUBMITTAL DATE: November 2002  
PROPOSED FINAL REPORT SUBMITTAL DATE: December 2002

#### 7.0 TEST PROTOCOL

The test protocol which follows is based on OECD Guideline for Testing of Chemicals, Method 203, entitled: Fish, Acute Toxicity Test (OECD, 1992).

#### 8.0 TEST SUBSTANCE

The Sponsor will provide characterization of the test substance's physical and chemical properties, toxicological hazard, and safe handling procedures. This information, as well as any known toxicity values, will guide the technical staff in handling and testing the test substance.

The Sponsor will be responsible for all test substance purity, stability, and characterization data as specified in Section 6.2 of OECD Principles of Good Laboratory Practice.

#### 9.0 DILUTION WATER

The dilution water will be laboratory freshwater with a hardness of 10 to 250 milligrams per liter (mg/L) as calcium carbonate (CaCO<sub>3</sub>) and an initial pH of 6.5 to 8.0.

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#### 10.0 TEST SUBSTANCE ADDITION/PREPARATION

All calculations of nominal test concentrations will be based on a whole product basis. If toxicity is not observed at or below the maximum solubility of the test substance in the dilution water, the highest test concentration definitively tested will be the maximum solubility of the test substance in the dilution water or 100 mg/L, whichever is lower.

#### 11.0 TEST ORGANISM

##### 11.1 Species

Rainbow Trout (*Oncorhynchus mykiss*)

##### 11.2 Source

Trout will be obtained from a commercial supplier.

##### 11.3 Size

Fish will be uniform in size; that is, the longest fish will be no more than twice the standard length of the shortest fish. Fish used in the test should range between 4 and 6 centimeters in total length.

##### 11.4 Acclimation/Holding

Fish will be gradually acclimated to within 2 degrees Celsius (°C) of the test temperature and to 100-percent dilution water. Fish will be maintained in the laboratory for a minimum of 12 days, 7 days in 100-percent dilution water, before starting the test. A group of fish will not be used if >10 percent of the population dies within a 7-day period. If mortality is between 5 and 10 percent of the population during the 7-day period immediately prior to test initiation, acclimation will be extended an additional 7 days. Fish will be acceptable if mortality of the population is <5 percent during the 7-day period prior to test initiation. Fish will be fed up until 24 hours before the test begins but will not be fed after that time or during the test.

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## 12.0 PROCEDURE

### 12.1 Experimental Design

Two toxicity tests will be conducted, a range-finding and either a limit test or a definitive test. The range-finding test is an abbreviated toxicity test employing widely spaced test substance concentrations to define the approximate concentration range within which the test substance produces a gradient from nontoxic to acutely toxic effects. If no toxicity results from exposure at 100 mg/L, a limit test may be performed at 100 mg/L to demonstrate that the median lethal concentration ( $LC_{50}$ ) is greater than this concentration. If toxicity is observed at 100 mg/L or below, the results of the range-finding test will guide selection of concentrations for the definitive test, the purpose of which is to provide a precise estimate of the 96-hour  $LC_{50}$  of the test substance.

The definitive test will consist of one or more control treatments and a geometric series of at least five test substance concentrations, unless a limit test is to be conducted. The concentration in each treatment, except for the highest concentration and the control(s) will be approximately 50 percent of the next higher one.

The test chambers will be glass and of a size sufficient to meet the loading restriction (i.e., loading will not exceed 1.0 grams of fish per liter of solution in the test chamber at any one time). All test chambers will be cleaned prior to the test with detergent and rinsed in sequence with clean water, 10-percent hydrochloric acid, deionized water, acetone, and deionized water. Prior to use, all test chambers will be rinsed with dilution water. A minimum of 7 fish will be randomly assigned to each test chamber. A minimum of two replicates will be utilized for each test concentration and the control(s) unless only a limit test is required and then the control and test concentration (usually 100 mg/L) will be conducted in triplicate. Test concentrations for the standard multiple concentration definitive test will be selected with the desired goal of obtaining at least one treatment which kills or affects more than 65 percent of the fish and one treatment (not the control) which kills or affects less than 35 percent of the exposed fish. The test will be

conducted for 96 hours, commencing when the fish are first exposed to the test substance.

The test will possess a control treatment consisting of the same dilution water, conditions, procedures, and organisms used in the other treatments, except that none of the substance being tested will be added to the dilution water. If any carrier other than water is present in any of the test chambers, a carrier (or solvent) control will be maintained concurrently. The carrier control will possess the greatest concentration of carrier present in any of the treatments. If a solvent/carrier is utilized, the concentration of solvent will not exceed 100 microliters/L.

#### **12.2 Temperature and Lighting**

Temperature will be regulated to maintain  $15 \pm 1^\circ\text{C}$ . A 16-hour light and 8-hour dark photoperiod will be maintained.

#### **12.3 Diet**

Fish will not be fed during the test.

#### **12.4 Dissolved Oxygen Concentration**

The dissolved oxygen concentration in the dilution water at test initiation should be  $\geq 90$  percent of saturation. The dissolved oxygen concentrations in the control(s) and all exposure solutions should remain above 60 percent of saturation during the test. Test solutions may be aerated during the test if it does not lead to a significant loss of test substance.

#### **12.5 Water Quality**

Specific conductivity, total alkalinity, and total hardness of the dilution water will be measured at test initiation. The temperature of the water bath will be continuously monitored using a minimum/maximum thermometer and the diurnal temperature range recorded daily. Temperature, dissolved oxygen concentrations and pH will be measured in all test concentrations and the control(s) once per day throughout the test in all test concentrations in which there are

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surviving fish. If 100-percent mortality is observed in a test concentration, water quality data will be recorded at that time and the concentration will be discontinued.

#### 12.6 Biological Data

Observations of mortality, moribundity, and behavior will be recorded and reported for all test concentrations. Observations will be made at 24, 48, 72 and 96 hours. Dead fish will be removed when observed.

#### 12.7 Chemical Analysis

Concentrations of the test substance will not be analytically confirmed. All concentrations will be reported as nominal.

#### 13.0 CALCULATIONS

Test results will be used to calculate the time dependent  $LC_{50}$  values (the calculated concentrations of the test substance which cause mortality in 50 percent of the test populations of fish at specified times of exposure). The 24-, 48-, 72- and 96-hour  $LC_{50}$  values will be calculated, when possible. Results will be calculated and reported on the basis of nominal test concentrations. The  $LC_{50}$  values will be calculated using a computer program based upon published methods for calculating  $LC_{50}$  values (Stephan, 1977).

#### 14.0 QUALITY CRITERIA

The test will not be valid if mortality in any control treatment exceeds 10 percent during the 96-hour test. Any deviations from recommended water quality or test conditions that may affect the toxicological response will be evaluated by the Study Director for their possible impact on the validity of the test.

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### 15.0 REPORT

The report will be a typed document describing the results of the test and will be signed by the Study Director and the Quality Assurance Unit. It will include, but not be limited to, the following information:

1. Name of test, investigator, and laboratory;
2. A detailed description of the test substance, including its source, lot number, and identity and concentrations of any solvents or other additives used;
3. The source of the dilution water, its chemical characteristics, and a description of any pretreatment;
4. Species tested, age, life stage, source, history, diseases observed and treatments, acclimation procedure, and diet;
5. A description of the experimental design and the test chambers, the depth and volume of solution in the chambers, the method for commencing the test, the number of fish per treatment, the loading, lighting conditions;
6. A description of any aeration performed on test solutions before or during the test;
7. Definition of the response criterion used to determine effect and a summary of general observations of other responses of the fish to the test substance;
8. Percentage of fish that died or showed the effect in all treatments, including the control(s);
9. When applicable, the calculated  $LC_{50}$  values, 95-percent confidence intervals, slope of the concentration/mortality response curves, and a reference to the method used to calculate these values; a no-observed-effect concentration (NOEC) will also be reported, if possible;
10. All pH, dissolved oxygen concentrations, total hardness, total alkalinity, specific conductivity and temperature measurements, and all visual observations of test solutions;
11. Dates encompassed by test;
12. List of all scientific personnel involved in the study;

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13. The storage location of all raw data and the final report;
14. Reference to the protocol and any amendments to or deviations from the protocol;
15. A statement signed by the Study Director which specifies that the study was conducted in accordance with published OECD Principals of Good Laboratory Practices; and
16. A statement signed by the Quality Assurance Unit that specifies the dates inspections were made and findings reported to management and to the Study Director.

#### 16.0 REFERENCES

OECD. 1992. Fish, Acute Toxicity Test. OECD Guideline for Testing of Chemicals, Method 203.

Stephan, C.E. 1977. Methods for Calculating an LC50. In: F.L. Mayer and J.L. Hamelink, eds., Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, American Society for Testing and Materials, Philadelphia, Pennsylvania. pp. 65-84.

#### 17.0 GOOD LABORATORY PRACTICES (GLP)

All test procedures, documentation, records and reports will comply with OECD's Principals of Good Laboratory Practices. To this end, Quality Assurance will, at random, select and audit at least one phase of the test while in progress. Quality Assurance will audit the final report against the raw data, protocol and standard operating procedures to assure the accuracy and integrity of the information presented. A statement, signed by Quality Assurance, attesting to the audits conducted will be included in the final report.

#### 18.0 PROTOCOL AMENDMENTS AND DEVIATIONS

All changes (i.e., amendments and deviations) of the approved protocol plus the reasons for the changes will be documented in writing and approved by the Study Director. All amendments will be signed and dated by both the Study Director and the Sponsor Representative and maintained with the protocol.

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### 19.0 RECORDS

Upon finalization of the report by the Study Director, all raw data generated in the conduct of the test, the protocol, pertinent written correspondence, and the final report will be temporarily archived at Toxikon Corporation. Following acceptance of the report by the Sponsor, all raw data, the protocol and written correspondence will be transferred to the Sponsor for permanent archiving at the Sponsor's expense.

### 20.0 TEST SUBSTANCE DISPOSAL

After acceptance of the final report by the Sponsor, Toxikon Corporation will return the remaining test substance to the Sponsor or arrange for proper disposal at the Sponsor's direction and expense.

### 21.0 CONFIDENTIALITY

Statements of confidentiality will be agreed upon prior to study initiation.

### 22.0 APPROVAL OF STUDY PROTOCOL

SPONSOR REPRESENTATIVE: *Patricia Anderson* DATE: 10/14/02

STUDY DIRECTOR: *Scott Olsen* DATE: 12/04/02

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JUPITER, FLORIDA

Protocol Amendment 1

**TITLE:** CT-756-02: Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions.

**STUDY NUMBER:** 02J0053b

**TEST SUBSTANCE:** CT-756-02

**STUDY SPONSOR:** Cytec Industries  
Five Garret Mountain Plaza  
West Paterson, New Jersey 07424  
TEL: (973) 357-3375 FAX: (973) 357-3057

**SPONSOR REPRESENTATIVE:** Patti Vernon

**Changes to test protocol**

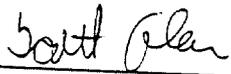
1. Nominal test concentrations selected for the definitive test will be 0.016, 0.080, 0.40, 2.0 and 10 mg wm/L.
2. The concentration in each treatment, except for the highest concentration and the control(s) will be approximately 80 percent of the next higher one.

**Reasons for changes to the test protocol**

1. The test concentrations for the definitive test must be specified in the test protocol per Annex 2 OECD Principles of GLP Guidelines.
2. Definitive test concentrations are selected with the desired goal of obtaining at least one concentration that kills or affects more than 65% of the fish and one treatment which kills or affects less than 35% of the exposed fish. Based on the results of the range-finding test, in which toxicity occurred over a broad range of test concentrations, definitive test concentrations had to be more widely spaced in order to achieve these goals.

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**APPROVAL**

  
\_\_\_\_\_  
Scott Glover  
Study Director  
Toxikon Corporation

2/03/03  
Date

  
\_\_\_\_\_  
Patti Vernon  
Sponsor Representative  
Cytec Industries

1/30/03  
Date

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